Accepted number	662-05-E-3741
Study number	93741

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FINAL REPORT

A 96-hour Acute Toxicity Study of

March 22, 2006

Chemicals Evaluation and Research Institute, Japan

STATEMENT

Sponsor

Title A 96-hour Acute Toxicity Study

Study number 93741

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Study No. 93741, issued on March 22, 2006).

Date September 20, 2006

Study Director

GLP STATEMENT

Sponsor

Title A 96-hour Acute Toxicity Study of

Study number 93741

This study was performed in compliance with:

(1) "Standard Concerning Testing Facility Relating to New Chemical Substances" (November 21, 2003; No. 1121003, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 17, 2003, No. 3, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121004, Environmental Policy Bureau, Ministry of the Environment)

(2) "OECD Principles of Good Laboratory Practice" (November 26, 1997)

This final report reflects the raw data accurately and it has been confirmed that the test data are valid.

Date March 22, 2006

Study Director Signed in original

QUALITY ASSURANCE STATEMENT

Sponsor

Title A 96-hour Acute Toxicity Study of

Study number 93741

It has been assured that the final report accurately describes the test methods and procedures, and that the reported results accurately reflect the raw data of the study.

The inspections of this study were carried out and the results were reported to the Study Director and the Test Facility Management by Quality Assurance as follows.

Item of inspection	Date of inspection	Date of report to Study Director and Test Facility Management
Study plan draft	February 9, 2006	February 9, 2006
Study plan	February 9, 2006	February 9, 2006
Start of the exposure and	February 13, 2006	February 17, 2006
after the exposure	February 17, 2006	February 17, 2006
Raw data and final report draft	March 18, 2006	March 18, 2006
Final Report	March 22, 2006	March 22, 2006

Date March 22, 2006

Quality Assurance Unit, Head Signed in original

CONTENTS

SUMMARY

A 96-hour Acute Toxicity Test of

<Test conditions>

- Test substance

- Test organism
  Medaka (*Oryzias latipes*)

- Exposure duration
  96 hours

- Test concentrations
  100 mg/L and a control

- Replicate
  2 vessels/exposure level

- Number of organisms
  10 fish/test level (5 fish/vessel)

- Dilution water
  Dechlorinated tap water

- Type of test
  Semi-static regime (renewal after 48 hours)

- Preparation of test solution
  The test solution was prepared by stirring it after adding the test item supplied by the sponsor to dilution water.

- Volume of test solution
  5 L/test level (2.5 L/vessel)

- Water temperature
  24±1℃

- Light condition
  Room lamp, 16-hour light/8-hour dark

- Feeding
  No feeding

- Aeration
  Conducted

- Analysis of concentration of test item in test solution
  HPLC analysis (at the start, before and after the renewal and the end of the exposure)

<Results>

- Concentration of test item in test solution (Percentage of nominal concentration)
  - At the start of the exposure and after the renewal 101 and 102%
  - Before the renewal and at the end of the exposure 103 and 102%

- 96-hour LC50 (Median Lethal Concentration)
  - >100 mg/L

(The above-mentioned concentration is based on nominal concentration.)

1. Title

A 96-hour Acute Toxicity Study of

2. Sponsor

Name

Address

3. Testing facility

Name

Address

4. Objective

The objective of this study is to determine the acute effects of the test item on fish.

5. Test method

The study was performed according to the following test methods.

(1) Fish, Acute Toxicity Test stipulated in the "Testing Methods for New Chemical Substances" (November 21, 2003; No. 1121002, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 13, 2003, No. 2, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121002, Environmental Policy Bureau, Ministry of the Environment)

(2) Fish, Acute Toxicity Test stipulated in the OECD Guidelines for Testing of Chemicals, Section 2 : Effects on Biotic Systems, 203, 1992

6. Applied GLP

This study was performed in compliance with:

(1) "Standard Concerning Testing Facility Relating to New Chemical Substances" (November 21, 2003; No. 1121003, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 17, 2003, No. 3, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121004, Environmental Policy Bureau, Ministry of the Environment)

(2) "OECD Principles of Good Laboratory Practice" (November 26, 1997)

7. Dates

1)	Study initiation date	February 9, 2006
2)	Experimental starting date	February 13, 2006
3)	Experimental completion date	February 17, 2006
4)	Study completion date	March 22, 2006

8. Storage of test item, raw data, etc.

1) Test item

The test item* supplied by the sponsor is sealed in a storage vessel and stored in archives in : ten years after the receipt of notice specified under Clause 1 or Clause 2 in Article 4, Clause 2 or Clause 3 or Clause 8 in Article 4-2, and Clause 2 in Article 5-4 or Clause 2 in Article 24 or Clause 2 in Article 25-3 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of the test material after the storage period is discussed with sponsor. If it is not stable for the storage period, it is stored as long while it is kept stable and it is disposed with approval of sponsor.

* It is stored as the common sample for storage of three tests

2) Raw data and materials

Raw data, the study protocol, documents concerning the study presented by the sponsor, the final report and necessary materials are stored in archives in Kurume Laboratory for ten years after the receipt of the notice specified under Clause 1 or Clause 2 in Article 4, Clause 2 or Clause 3 or Clause 8 in Article 4-2, and Clause 2 in Article 5-4 or Clause 2 in Article 24 or Clause 2 in Article 25-3 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of raw data and materials, etc. after the storage period is discussed with the sponsor.

9. Personnel

Study Director :

Study Personal

Biology :

Analytical Chemistry :

10. Approval of final report

Study Director Date March 22, 2006

Signature Signed in original

11. Test item

following name etc. in this final report.

11.1 Test item

The following are within the test item information provided by the sponsor.

1) Name

2) Name (abbreviation)

3) Rational formula etc.

(1) Rational formula

(2) Molecular formula

(3) Molecular weight

11.2 Test item supplied by the sponsor

The following are within the test item supplied by the sponsor information provided by the sponsor.

1) Lot Number

RS4-56

2) Purity

99.5%(w/w)

3) Name and content of impurity

Water 0.5%(w/w)

4) Physicochemical properties etc.

(1) Appearance at normal temperature

White solid

(2) Melting point

(3) Soluble property

Oil and water soluble

(4) Stability

Stable under room temperature

Stable in water, DMSO and acetone

5) Supplier

11.3 Confirmation of test item supplied by the sponsor

It was confirmed that infrared (IR) spectrum of the test item provided by the sponsor coincided with IR spectrum analyzed in .

11.4 Storage condition and confirmation of stability under the storage condition

1) Storage condition

The test item supplied by the sponsor was kept in a dark place at room temperature during the test period.

2) Confirmation of stability

The stability of the test item during the test period was confirmed by no alteration in the IR spectra of the test item before the experimental start and after the experimental completion.

12. Test materials and methods

1) Test organism

(1) Species

Medaka (*Oryzias latipes*)

(2) Reason for selection of species

Species recommended in the test guidelines.

(3) Size

Total length 2.3±1.2 cm

The regulated size of test organism set to 5.(1) was applied.

(4) Supplier

In-house production.

(5) Acclimation

The test organisms hatched out on June 20, 2005 were acclimated for 31 days by flow-through condition under the same water quality (dechlorinated tap water), temperature (24±1℃), photoperiod (16-hour light/8-hour dark) as test condition. The mortality was 0% during the 7 days before the start of the exposure. The test organism at the start of the exposure was about 7-month-old fish. The test organisms were not treated with a medicament for external disinfection. The test organisms were fed the feed mixture for carp (2C), and not fed for 24 hours before the start of the exposure. Dissolved oxygen concentration in breeding water during acclimation was kept not less than 80% of air saturation value. A 96-hour acute toxicity test of $CuSO_4$ $5H_2O$ (Reagent chemical, Wako Pure Chemical Industries, Ltd.) to confirm reproducibility of the test system was carried out on January 23-27, 2006, and the 96-hour LC50 was 0.549 mg/L. This value was within the stipulated range (mean±2S.D. : 0.118 to 0.992 mg/L) [mean±S.D. : 0.555±0.218 mg/L(n=35)] to background data in All of the values shown above for the reference substance were converted into $CuSO_4$ value.

(6) Allocation to the test groups

Medaka were allocated at random to each test group.

2) Dilution water

Dechlorinated tap water, aerated sufficiently and controlled temperature, was used. Some chemical characteristics of the dilution water are listed in Appendix 1.

3) Test apparatus and equipment

(1) Test apparatus

Test vessel : 3.0 L Glass tank (Diameter: 16 cm, Depth: 17 cm)

The test vessels were covered with lid in order to prevent dust, and volatilization of the test solution.

(2) Test equipment

Water bath : Plastic tank

Warming/cooling unit (Type HCA250, Sato craft)

4) Test conditions

(1) Conditions of exposure

①Type of test

The test organisms were exposed to the test solution containing the test item.

The test solutions were renewed after exposure for 48 hours, as semi-static regime.

②Exposure duration

96 hours

③Test concentration

One exposure level (the maximum concentration on applied test guidelines : 100 mg/L) was used in the test from the results of preliminary tests. The test concentration was expressed as value corrected by the purity (99.5%) of the test item. The results of preliminary tests are shown in additional data.

④Control

Only the dilution water was used as the control.

⑤Number of organisms

10 fish/test level (5 fish/vessel)

⑥Volume of test solution

5 L/test level (2.5 L/vessel)

(2) Conditions of test environment

①Water temperature

24±1℃

②Dissolved oxygen concentration

This study was performed in the condition where dissolved oxygen concentration was 60% or more of the saturate concentration at the water temperature used. Gentle aeration was used for the test during the exposure.

③pH

This study was performed without adjusting pH.

④Light

16-hour light/8-hour dark photoperiod daily with room lamp

⑤Feeding

Test organisms were not fed during the exposure.

5) Preparation of test solution

Correction with the purity (99.5%) was applied to the preparation of the test solution.

Desired amount of test item supplied by the sponsor was added to the dilution water in the test vessel and the mixture was stirred to prepare the test solution. Amount of test item supplied by the sponsor for preparation in each exposure level is shown below.

Test level (mg/L)	Amount of test item supplied by the sponsor [mg/2.5 L(× replicate)]
Control	–
100	251.3 (×2)

6) Observation and measurements

(1) Observation of test organisms

Fatality and visible abnormality were observed at 3, 24, 48, 72 and 96 hours after the start of the test in each exposure level. A fish was considered as dead if the observable motion (mouth and opercle motion etc.) were not observed and touching of the caudal peduncle with glass rod produced no reaction.

(2) Total length and body weight of test organism

The test organisms in the control group were used for measuring total length and body weight after the end of the exposure.

(3) Appearance of test solution

Appearance of the test solutions were observed at the start of the exposure and before the renewal after 48 hours.

(4) Water quality

Dissolved oxygen concentration, pH and water temperature of the test solutions were measured at the start of the exposure, before and after the renewal, and the end of the exposure. Also 24 and 72 hours after, water qualities were measured. The measurement was carried out for one test vessel in each level. The dissolved oxygen concentration measurements were carried out on an oxygen meter (YSI Model 58, Yellow Springs Instruments.). The pH measurements were carried out on a pH meter (Model HM-21P, DKK-TOA). The water temperature measurements were carried out on a calibrated red alcohol thermometer of glass stick type.

(5) Concentration of test item in test solution

The concentration of the test substance in the test solution was measured at the start of the exposure, before and after the renewal, and at the end of the exposure. The test solution for analysis was taken out from the middle layer of the test solution in the test vessels in each level and mixed. The concentration of the test item was analyzed by high-performance liquid chromatography (HPLC). Method of analysis and result of measurement of test item concentration are shown in Appendix 2, and analytical calibration curve and chromatograms are shown in Appendix 3.

(6) Solubility in dilution water

Solubility in dilution water was not measured at this study because the solubility was greater than 100 mg/L.

7) Calculating method of LC50*[1]

The LC50 value was estimated as "> test concentration" since no mortality was observed in the present exposure level.

The results of the study were estimated based on nominal concentrations because the measured concentrations of the test item in the test solution during the exposure were kept within the range of ±20% of the nominal concentrations.

*[1] LC50 (Median Lethal Concentration) is the concentration which causes 50% mortality of tested population during the exposure.

8) Study validity

(1) The mortality in the control should not exceed 10 %.

(2) Dissolved oxygen concentration of the test solution must be at least 60 % of the air saturation value at the water temperature in the test during the exposure.

9) Treatment of numerical values

Values were rounded off in accordance with JIS Z 8401 rule B.
(JIS ; Japanese Industrial Standards)

13. Results and consideration

1) Mortality

No mortality of test organism was observed in the exposure level during the exposure. Cumulative mortalities at each observation time are shown in Table 1. Cumulative mortality in the control at the end of the exposure was 0%, which meets the criterion for the validity of the test (i.e. less than 10%).

2) Observed abnormal response

No abnormal response was observed in the control. The following results of observation were based on the comparison with the control. No abnormal responses were observed in the exposure level during the exposure. The result of the observation for abnormal response during the exposure is shown in Table 2.

3) Size of test organism [Mean±Standard deviation (n=10)]

Total length	2.8±0.18 cm
Body weight	0.17±0.035 g

4) Observation and measurement of test solution

(1) Appearance of test solution

The test solutions were clear and colorless at the start of the exposure. The appearance kept until the end of the exposure.

(2) Water quality of test solution

The measured values of DO, pH and water temperature during the exposure ranged from 7.7 to 8.6 mg/L, 7.4 to 7.6 and 23.6 to 24.4℃, respectively. Water qualities of the test solutions are shown in Table 3-1, 3-2, and 3-3. The measured values of DO met the criterion for the validity of the test (not less than 60% of air saturation value*[2] at the water temperature in the test).

*[2] Saturated dissolved oxygen concentration (23 - 25℃) : 8.39 - 8.11 mg/L
(JIS K 0102)

(3) Concentration of test item in test solution

The measured concentrations of the test item in the test solution were 101 and 102% of the nominal concentration at the start of exposure and after the renewal, and 103 and 102% before the renewal and at the end of the exposure. The measured concentration of the test item was kept within ±20% of the nominal concentrations during the exposure. The results of the measured concentrations of the test item are shown in Appendix 2.

5) LC50

Both of the 48- and 96-hour LC50s of >100 mg/L.

The LC50s at every 24 hours are shown in Table 4.

6) Consideration

The present study was conducted as a limit test to confirm that the test item has no effects on the test organisms at the maximum concentration on applied test guidelines (100 mg/L). The measured concentrations of the test solution were kept within the range of ±20% of the nominal concentration, and the environmental conditions were also within the suitable range. Therefore, it is concluded that the results in this study were appropriately obtained in the suitable conditions where the present test was performed according to the test guidelines.

14. Factors that affected the reliability of the test results

No adverse effect on the reliability of this study was observed.

Table 1 Cumulative mortality

Nominal concentration (mg/L)	Cumulative mortality (%)				
	3 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
100	0	0	0	0	0

Table 2 Observed abnormal response

Nominal concentration (mg/L)	Result of observation (Left column: Number of affected fish/Total survival number, Right column: Symptom detail)									
	3 hours		24 hours		48 hours		72 hours		96 hours	
Control	0/10	–	0/10	–	0/10	–	0/10	–	0/10	–
100	0/10	–	0/10	–	0/10	–	0/10	–	0/10	–

–: Normal (No abnormal response)

Table 3-1 Dissolved oxygen concentration of test solutions

Nominal concentration (mg/L)	0 hour	24 hours	48 hours		72 hours	96 hours
	At the start		Before the renewal	After the renewal		At the end
Control	8.6	8.1	7.8	8.6	7.9	7.7
100	8.6	8.0	7.7	8.5	7.9	7.7

Unit : mg/L

Table 3-2 pH of test solutions

Nominal concentration (mg/L)	0 hour	24 hours	48 hours		72 hours	96 hours
	At the start		Before the renewal	After the renewal		At the end
Control	7.6	7.4	7.5	7.6	7.5	7.5
100	7.6	7.5	7.5	7.5	7.5	7.5

Table 3-3 Water temperature of test solutions

Nominal concentration (mg/L)	0 hour	24 hours	48 hours		72 hours	96 hours
	At the start		Before the renewal	After the renewal		At the end
Control	23.6	24.4	23.7	23.6	23.9	23.6
100	23.6	24.4	23.7	23.6	23.9	23.6

Unit : ℃

Table 4 LC50 to Medaka

Exposure duration	LC50 (mg/L)	95% confidence intervals (mg/L) (Slope of the dose-response curve)	Statistical procedure used for determination of LC50
24 hours	>100	— (—)	—
48 hours	>100	— (—)	—
72 hours	>100	— (—)	—
96 hours	>100	— (—)	—

—: Not obtained

Appendix 1

Water quality of dilution water

Water quality of dilution water (Sampling on January 11, 2006)

Parameter	Unit	Results	Lower limit of determination
Hardness (as $CaCO_3$)	mg/L	41.7	0.1
Suspended substance	mg/L	< 1	1
pH	—	7.9(19℃)	—
Organic carbon	mg/L	0.8	0.1
Chemical oxygen demand	mg/L	1.0	0.5
Free chlorine	mg/L	< 0.02	0.02
Ammonium nitrogen	mg/L	0.01	0.01
Cyanide	mg/L	< 0.01	0.01
Alkalinity	mg/L	36	1
Electric conductivity	mS/m	17.1	—
Organic phosphorous	mg/L	< 0.1	0.1
Alkyl mercury	mg/L	< 0.0005	0.0005
Total mercury	mg/L	< 0.0005	0.0005
Cadmium	mg/L	< 0.001	0.001
Hexavalent chromium	mg/L	< 0.02	0.02
Lead	mg/L	< 0.005	0.005
Arsenicum	mg/L	< 0.001	0.001
Boron	mg/L	0.07	0.02
Fluorine	mg/L	0.1	0.1
Iron	mg/L	< 0.01	0.01
Copper	mg/L	< 0.005	0.005
Cobalt	mg/L	< 0.001	0.001
Manganese	mg/L	< 0.01	0.01
Zinc	mg/L	< 0.01	0.01
Aluminum	mg/L	0.063	0.001
Nickel	mg/L	< 0.001	0.001
Silver	mg/L	< 0.0001	0.0001
Sulfate ion	mg/L	16.2	0.1
Chloride ion	mg/L	14	1
Sodium	mg/L	13.4	0.01
Potassium	mg/L	3.5	0.01
Calcium	mg/L	11.2	0.01
Magnesium	mg/L	3.3	0.01
1,2-dichloropropane	mg/L	< 0.0001	0.0001
Chlorothalonil	mg/L	< 0.0001	0.0001
Propyzamide	mg/L	< 0.0001	0.0001
Chlornitrofen	mg/L	< 0.0001	0.0001
Simazine	mg/L	< 0.001	0.001
Thiobencarb	mg/L	< 0.0001	0.0001
Diazinon	mg/L	< 0.0001	0.0001
Isoxathion	mg/L	< 0.0001	0.0001
Fenitrothion	mg/L	< 0.0001	0.0001
EPN	mg/L	< 0.0001	0.0001
Dichlorvos	mg/L	< 0.0001	0.0001
Iprobenfos	mg/L	< 0.0001	0.0001
PCB	mg/L	< 0.0005	0.0005

Appendix 2

Method of analysis and result of measurement of test item concentration

1. Method of analysis of test item concentration

1) Pretreatment of test solution

The test solutions sampled were used as the samples for analysis after no treatment.

2) Method of analysis

The pretreated samples for analysis were quantitatively analyzed by high-performance liquid chromatography (HPLC) under the following conditions to determine the concentration of the test item. The concentration of the test item in the samples for analysis was proportionally calculated by comparing each peak area of the test item on the chromatogram with that of a standard solution of known concentration. The obtained some chromatograms are shown in Appendix 3.

Analytical conditions

Instrument	High-performance liquid chromatograph SHIMADZU LC-2010A$_{HT}$
Column	L-column ODS (Chemicals Evaluation and Research Institute) 15 cm×2.1 mm I.D.
Column temp.	40℃
Eluent	Acetonitrile/10 mmol/L tetra-*n*-butylammonium phosphate solution* 55/45(v/v)
Flow rate	0.2 mL/min
Wave length	
Injection volume	5 μL
Sensitivity	
Detector	0.5 AU/V

* It was prepared from tap water treated with a ultra pure water system.

3) Preparation of standard solution

The standard solution to determine the concentration of the test item in the sample for analysis was prepared as follows. The standard solution was prepared with correcting by the purity (99.5%) of test item.

Test item supplied by the sponsor of 100.5 mg was precisely weighed with an electronic balance and dissolved in dechlorinated tap water to prepare 1,000 mg/L of test item solution. The standard solution of 100 mg/L was then prepared from this solution by dilution with dechlorinated tap water.

4) Calibration curve

The standard solutions of 10.0, 50.0, 100 and 200 mg/L were prepared for analysis by the same procedure as described in 3). These solutions were analyzed according to the quantitative analytical conditions described in 2). A calibration curve was drawn from the relationship between the concentrations of test item and the peak area on the chromatogram respectively, the quantitative correlation was confirmed. The calibration curve and regression equation are shown in Appendix 3. The determination-limit of the test item in the test solution was the lowest determination-limit (10.0 mg/L) of the standard solutions within the range of the calibration confirmed.

2. Results of the measurement

The results of the measured concentrations of the test item in the test solution are shown below.

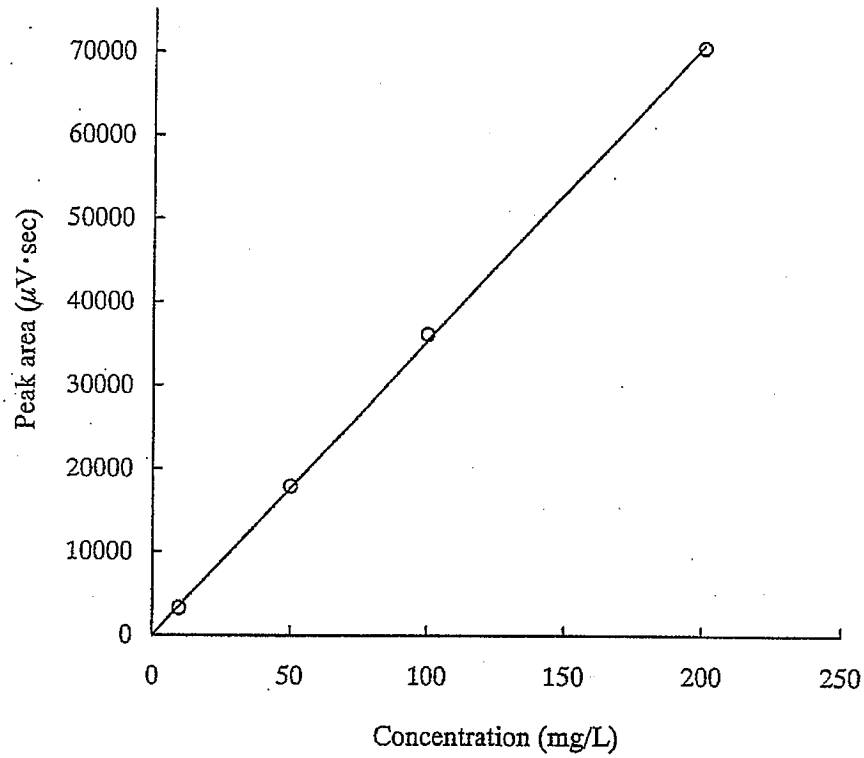
Appendix table 2-1. Measured concentrations of test item in test solutions

Nominal concentration (mg/L)	Measured concentration of test item (mg/L) (Parcentage of nominal concentration)				
	At the start	48 hours		At the end	Mean*
		Before the renewal	After the renewal		
Control	n.d.	n.d.	n.d.	n.d.	—
100	101 (101)	103 (103)	102 (102)	102 (102)	102 (102)

n.d. : Not determined (<10.0 mg/L)
* The value is expressed as geometric mean.

Appendix 3

Calibration curve and chromatograms



y = 354x
r = 1.00

Appendix figure 3-1. Calibration curve of EEA-NH4 by HPLC.

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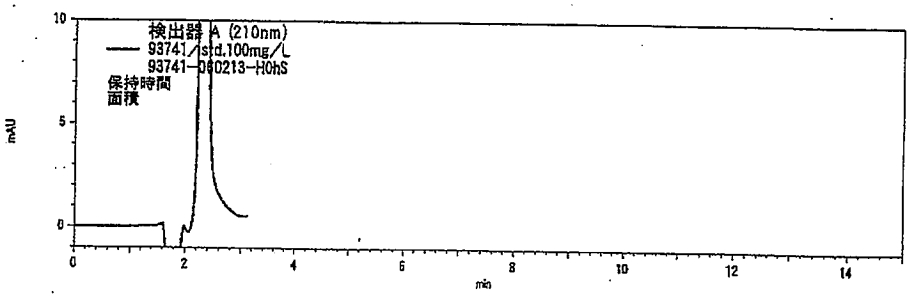
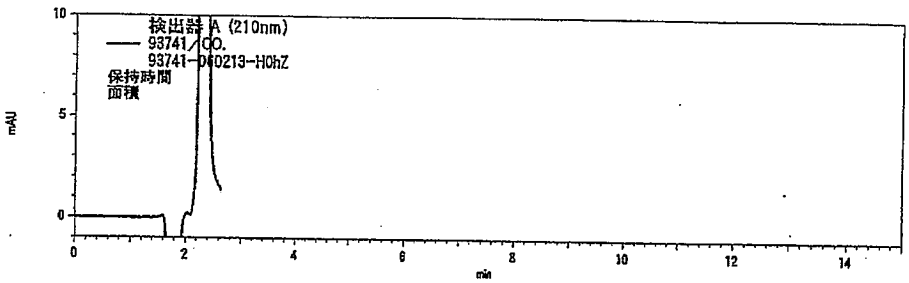
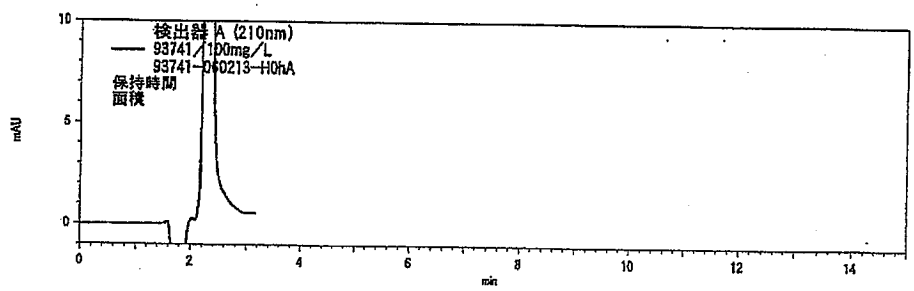
Standard solution 100 mg/L



Control



100 mg/L (Nominal concentration)



Date 2006 / 2 / 13 Name

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Appendix figure 3-2. Chromatograms for test solution at the start of the exposure.

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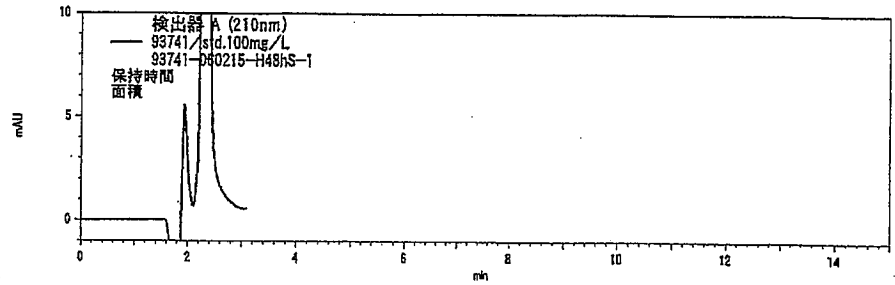
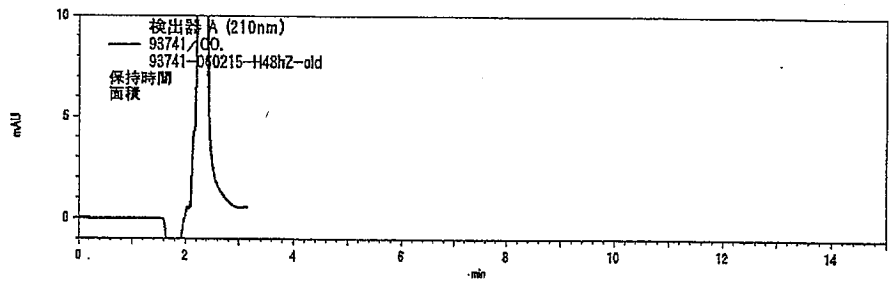
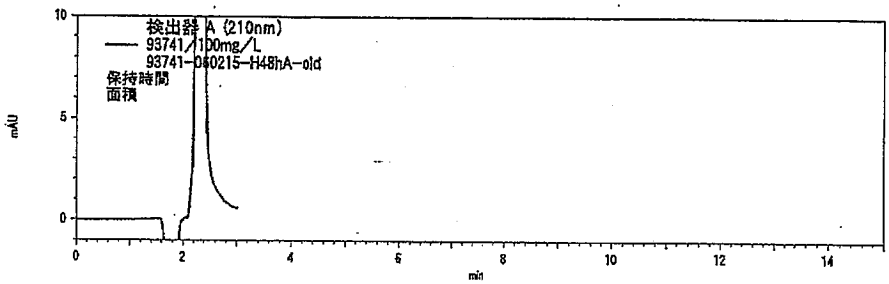
Standard solution 100 mg/L



Control



100 mg/L (Nominal concentration)



Date 2006 / 2 / 15 Name

Appendix figure 3-3. Chromatograms for test solution at 48 hours after the exposure (before the renewal).

Additional data

Results of preliminary tests

1. Solubility of test item in dilution water
   1) Examination
      (1) Method

Solubility of the test item was confirmed by mixing them the test item supplied by the sponsor and dilution water to produce concentration of 100 mg/L

(2) Result

The test solution was clear and colorless, and no insoluble material was observed. Therefore, it was concluded that the test item in dilution water at 100 mg/L was dissolved.

2) Summary

It was decided that solubility of the test item to dilution water was not measured in this study because the solubility in dilution water was greater than 100 mg/L.

2. Effect on test organism
   1) Preliminary test
      (1) Method

Desired amount of test item supplied by the sponsor was added to the dilution water in the test vessel to prepare the maximum concentration on applied test guidelines (100 mg/L). The mixture was stirred to prepare the test solution, and effect on the test organism was confirmed by exposing the organisms to the test solution.

(2) Result

Nominal concentration (mg/L)	Left column : Cumulative mortality (%) Right column: Existence of abnormal response (abnormalities : *, no abnormalities : −)									
	3 hours		24 hours		48 hours		72 hours		96 hours	
100	0	−	0	−	0	−	0	−	0	−

Type of test: Semi static method (Frequency of renewal ; 1 time/2 days)
Number of organisms/ Volume of test solution : 2 fish／1 L
Aeration: Not conducted

Mortality and effect was not observed to test organisms.

3. Test item concentration in test solution
   1) Method

The test item concentration in the test solution were measured at start of the exposure, after 24 and 48 hours in the same conditions as Preliminary test described in 2.1). The test with aeration was conducted to confirm effect of aeration for maintenance of the test item concentration.

2) Result

Nominal concentration (mg/L)	Measured concentration (mg/L) (Percentage of nominal concentration)		
	At the start	After 24-hour	After 48-hour
100	102	105	107
100 +aeration	105	106	107

With or without aeration, the test item concentrations were kept until 48 hours.